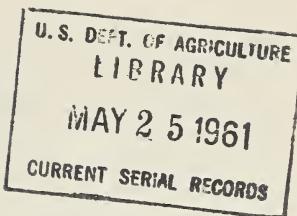


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ANTIOXIDANTS AND OTHER  
FEED ADDITIVES IN

# *Fish* *Diets* *for* *Minks*

EFFECT ON REPRODUCTION AND STEATITIS

Production Research Report No. 49

Agricultural Research Service  
UNITED STATES DEPARTMENT OF AGRICULTURE  
*in cooperation with* the Alaska Experiment Stations  
and the University of Alaska

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# ***Fish Diets For Minks:***

## **Effect on Reproduction and Steatitis**

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The search for economical feeds is of general importance in animal production but is of especial interest in mink feeding because of the rapid growth of the industry and the decrease in the traditional feed supply of horse meat.

Between 1950 and 1960, production of ranch-raised mink pelts in the United States increased from a little more than 1 million to over 5 million with an approximate value of over 90 million dollars.

Little is known about the food potential for fur animals of the vast amount of scrap fish and fish waste products of the large fishing industry of Alaska. An estimated 50,000 tons per year of waste salmon products is discarded by canneries, and there are large quantities of so-called bottom fish for which there is no market for human consumption. Basic knowledge of the feeding of these products—species, amounts, supplements needed—to obtain normal production of good quality fur would be of great benefit to the fur industry in other States as well as in Alaska.

To answer some of these questions, cooperative research has been conducted by the U.S. Department of Agriculture and the Alaska Experiment Stations, and the University of Alaska.

For some years salmon cannery waste has been fed in experimental rations to minks at the Experimental Fur Station, Petersburg, Alaska. This has been done in an effort to formulate a practical ration from this easily available and economical fish product. Development of steatitis (yellow fat disease), especially in growing kits fed salmon waste, has been an undesirable feature, as previously reported (4).<sup>1</sup> Steatitis also has been observed frequently on commercial fur farms when attempts have been made to use salmon cannery waste as a substantial part of the diet for minks. Signs and histopathology of this disease have been described in detail (2, 5).

The relatively high unsaturated fatty acid content of the salmon waste products is susceptible to decomposi-

<sup>1</sup> Italic numbers in parentheses refer to Literature Cited, p. 22.

tion with the forming of compounds that destroy available vitamin E in the mink diet. Addition of vitamin E in various carriers has reduced incidence of yellow fat in many feeding trials, but the levels fed did not give adequate protection.

The successful practice of using antioxidants in other livestock feeds, in addition to added vitamin E, suggested experimental work on feeding salmon waste supplemented with some of the compounds that are known to be effective fat stabilizers. Others (6) have pointed out the importance of including reproductive studies in experiments on feed additives that might result in chronic toxicity or other undesirable effects. This was considered in planning the work to be discussed here. The purpose of this report is to describe the rather extensive experiments conducted during a period of 5 years. The objectives of the experiments were: (1) to test the efficiency of various additives, including certain types of antioxidants, for controlling steatitis in minks, and (2) to establish a safe feeding level of an antioxidant that would not adversely affect breeding, reproduction, growth, or fur production.

The results and conclusions should be of interest in the general problem of feeding fish and other fat-containing feeds to minks. Also, at the present time (1960), the antioxidant butylated hydroxy toluene (BHT) is being tentatively permitted in other animal feeds and human food. Data on its cumulative or residual effects over extended periods on various animals is of interest. Some preliminary reports on the data given here have been made (1, 3).

## EXPERIMENTAL TECHNIQUE AND EQUIPMENT

### DIET MATERIALS

Salmon waste from pink salmon (*Oncorhynchus gorbuscha*), chum salmon (*O. keta*), and red salmon (*O. nerka*) was fed in the experiments. The waste consisted largely of heads, tails, fins, and viscera. It was collected at local canneries during summer months in 5-gallon cans with clinch lids, frozen in a commercial freezer, and stored in the Station cold storage until used.

The flounders (*Pleuronectidae* spp.) were purchased from local fishermen at various times throughout the year, frozen in the Station cold storage in pans holding approximately 50 pounds, and kept well glazed until fed.

For a description of other ration ingredients, see the Appendix, page 23.

### PROCEDURE

During each year of the 5-year period 1955-59, feeding trials were conducted with ranch minks to evaluate phenolic-type antioxidants and other additives in mink diets containing 50 to 80 percent waste from Alaska salmon canneries. All these feeding trials were designed as factorials using six or eight treatments, starting with equalized outcome groups of 12 to 16 female minks. The feeding trials were all begun early in the year at least a month before the breeding season. Animals were fed throughout the breeding, gestation, and suckling periods; one-half of the producing females and their kits, in most cases, were maintained on the diets until pelting, the following December.

This general procedure and others outlined in this section were followed in all experiments except in the special instances noted.

The animals in all trials were fed once daily between 3:00 and 5:00 p.m. on 5 by 7½ inch metal feeders inside each pen or at times on top of their wire pens. Each lot was full fed, and undue waste was avoided. Unconsumed feed was redistributed between 8:00 and 9:00 a.m. and picked up about noon each day.

Frozen salmon waste and flounders were removed from cold storage, thawed on the feed room floor, and ground and mixed with the other ingredients just prior to feeding. Since the supply of material from the previous year was usually exhausted by summer, feeding of currently collected frozen waste was started with the animals then on experiment and continued through the next experiment to the following summer months. From one to several weeks' supply of the dry feed ingredients—dry mix brewers' yeast, wheat germ meal, liver meal, antibiotic, and antioxidant—were mixed together by mechanical mixer and stored in a metal container until used.

#### HOUSING

All breeding animals were housed in a colony-type, fully-covered mink house. Animal pens were 1-inch square-mesh wire, 36 inches long, 21 inches wide, and 18 inches high, elevated about 4 feet above the ground. Each pen was equipped with an 8-ounce water pan and a 12 by 10 by 12 inch covered nest box. After weaning, at 50 days of age, the young animals were housed in a similar building in individual furring

pens 24 inches long, 15 inches wide, and 18 inches high. These pens were equipped with a 12-ounce water pan and an 8 by 8 by 11 inch insert-type nest box. Animals in each lot<sup>2</sup> were distributed to obtain as nearly comparable housing conditions as possible.

#### WEIGHING

Each female and her individual kits were weighed on the days the kits were 49 and 50 days of age. These weights are referred to as weaning weights. The young animals that remained on the experiments throughout the summer and fall months were weighed at 28-day intervals after late July or August until the end of the experiment in December.

#### BREEDING

Breeding attempts were started about the middle of March in accordance with a prearranged breeding schedule whereby one male was mated with two to five females. Second matings were attempted daily, starting on the seventh or eighth day after the first mating and, when obtained, the same animals were mated again the following day. The breeding season was ended the second week in April.

#### STATISTICAL STUDIES<sup>3</sup>

Chi-squared tests were made on the number of females producing, and

<sup>2</sup> The term "lot" is used throughout the text and tables to denote a group of animals fed a particular diet.

<sup>3</sup> The statistical designs used in these experiments were suggested by members of the staff of Biometrical Services. The statistical analyses were made by Dr. R. A. Damon of that Section.

the number of kits weaned in all five experiments. Tests of significance were made among the same types of treatments (i.e., single, double, or triple), and these were broken down into orthogonal comparisons where it appeared worthwhile to do so.

Similar analyses were made among the weaning weights, after first adjusting for difference in sex. As the females weighed less than the males, their weights were adjusted upward to a male basis before the analyses were made. The adjustment factors used for each of the experiments are shown in the Appendix, page 24. Since there were unequal numbers, a least squares analysis was made on the data. A preliminary analysis was made to determine whether significant differences existed among the treatment means and when such differences were found, the analyses were extended to make orthogonal

comparisons among the treatment means.

## FEEDING EXPERIMENTS WITH DIPHENYL-P. PHENYLENE DIAMINE (DPPD)

Two feeding-reproduction experiments were conducted with DPPD. All diets in these experiments contained 80 percent of salmon waste. The first was started February 1, 1955, and continued through to pelting in December. The second was for a similar period in 1956.

### EXPERIMENT 1

This experiment containing six lots of 15 females each was designed as a factorial in which the variables tested were the antioxidant DPPD, an antibiotic, and tocopherol. Composition of the diets is given in table 1.

TABLE 1.—DIETS: *Experiment 1*

Basal diet (all lots)		Parts
Chum salmon waste <sup>1</sup>		80
Dry mix No. 3 <sup>2</sup>		14
Wheat germ meal		2
Water		4
Total		100
Additives	Lot	Quantity
Tocopherol <sup>3</sup>	III, IV	1.2 lbs. per ton of feed.
Antibiotic <sup>4</sup>	IV, V, VI	1.6 lbs. per ton of feed.
Antioxidant <sup>5</sup>	II, III, V, VI	112 gm. per ton of feed.
Wheat germ oil <sup>6</sup>	Each lot	5 gm. per day.

<sup>1</sup> Pink salmon waste replaced the chum salmon waste from May 1 until the experiment ended.

<sup>2</sup> See Appendix, p. 23.

<sup>3</sup> A commercial grade labeled to contain 20,000 International Units of vitamin E per pound as alpha tocopherol acetate.

<sup>4</sup> Labeled to contain not less than 5 grams of chlorotetracycline (CTC) per pound.

<sup>5</sup> Diphenyl-p-phenylene diamine (DPPD).

<sup>6</sup> Wheat germ oil was added to the daily ration of each lot from Feb. 1 until Apr. 1.

## *Production and Growth of Kits*

The data on reproduction are given in table 2. Breeding was exceptionally good; 89 of the 90 females were bred. Results as measured by other criteria, including the number of females producing and number of kits weaned, were disappointing. This was most marked in lot VI, which received the triple treatment of tocopherol, antibiotic, and antioxidant. Of the 15 females started in this lot, only 7 produced and only 19 kits were weaned.

Significant differences occurred between lot VI and all other lots in the number of females producing and the number of kits weaned (table 3). However, no significant differences are indicated among the single or double treatments.

Analysis of variance of the weaning weights of the kits (table 4) shows no significant differences among the treatments, which indicates that the treatment variables had no effect on weaning weights of the kits.

TABLE 2.—PRODUCTION: *Effects of feed additives, tocopherol, DPPD, and CTC, Experiment 1*

Traits measured	Lot No. and additive						Total or aver- age, all lots
	I Control	II DPPD	III DPPD TOCOL	IV CTC	V CTC DPPD	VI CTC DPPD TOCOL	
Females:							
Started on experiment							
number	15	15	15	15	15	15	90
Mated	14	15	15	15	15	15	89
Died prior to whelping							
number	0	0	1	0	0	0	1
Producing	13	11	12	12	10	7	65
Litters destroyed	3	0	1	3	1	2	10
Kits:							
Total:							
At 10 days of age							
number	49	48	39	43	39	19	237
Died between 10 days							
and weaning <sup>1</sup>							
number	7	3	3	3	1	0	17
Weaned	42	45	36	40	38	19	220
Average:							
At 10 days per female							
started	3.26	3.2	2.78	2.86	2.6	1.26	2.66
Weaned per female							
started	2.8	3.0	2.57	2.66	2.53	1.26	2.47
Weaned per producing							
female	3.23	4.09	3.0	3.33	3.16	2.71	3.38
Weaning weight at 50							
days of age							
pounds	.658	.697	.755	.701	.711	.776	.671

<sup>1</sup> Includes several whole litters destroyed between 10 days of age and weaning.

Excludes 1 female that died prior to whelping.

TABLE 3.—*Chi-square studies of effects of treatment variables on production, Experiment 1*

Comparisons	Degrees of freedom	X <sup>2</sup> tests of significance among treatments <sup>1</sup>	
		Number of females producing	Number of kits weaned
Control vs. treated-----	1	1. 872	0. 931
DPPD vs. CTC-----	1	. 166	. 294
Tocopherol-DPPD vs. CTC-DPPD-----	1	. 664	. 054
Single vs. double treatments-----	1	. 083	. 761
Single and double vs. triple treatment-----	1	4. 802*	9. 676**

<sup>1</sup> 1 asterisk indicates significance at the 5-percent level; 2 asterisks indicate significance at the 1-percent level.

TABLE 4.—*Analysis of variance of weaning weights of kits, Experiment 1*

Source of variation	Degrees of freedom	Sums of squares	Mean squares
Replicates-----	14	2. 5666	0. 1833**
Treatments-----	5	. 0977	. 0195
Error-----	199	3. 6398	. 0183

<sup>1</sup> 2 asterisks indicate significance at the 1-percent level.

#### *Incidence of Steatitis*

Because of the potential losses of many kits from steatitis in these experiments, at the time the plans were made it was decided that when the probability of large losses became evident in any lot the kits would be removed from the steatitis-producing diet. For this reason, after several death losses and symptoms of steatitis in lots I and IV, the remaining animals were removed from the fish waste diets. Some further losses from steatitis occurred in these two lots in the next few days, and the rest of the animals were fed a non-steatitis-producing diet to pelting in December. The carcasses were then examined for steatitis along with the carcasses from lots that had received

fish waste diets and DPPD throughout the feeding period.

None of the 48 kits from the 4 lots that received DPPD showed any gross symptoms of steatitis, whereas 9 of 30 kits in lots I and IV fed through most of July on the fish waste with no DPPD were affected. Thus, regardless of the inconclusive results on reproduction, further experimental work with DPPD appeared to be justified.

#### EXPERIMENT 2

Eight lots of 12 females each were fed in an experiment of factorial design. The variables again were the antioxidant DPPD, an antibiotic, and tocopherol.

## Production and Growth of Kits

Four of the lots in this experiment received DPPD (table 5). It was fed to the females at a level of 224 grams per ton of feed from February 1 until March 12, then reduced to the 112-gram level. Some of the females distributed throughout the eight lots were obtained from litters that had been fed DPPD the previous year, and this was taken into consideration in equalizing the outcome groups at the beginning of the experiment.

Reproduction failure and early loss of litters appeared to be abnormally high among the animals receiving DPPD (table 6). Only 65 of the 96 females started on test produced litters, and 9 of these destroyed or lost their litters before weaning. Furthermore, 38 of the 40 females that either did not produce

or lost their litters received DPPD either in 1955 or 1956.

Statistical treatment of the data from Experiment 2 on the number of females producing (table 7) shows that the only orthogonal comparison indicating harmful effects of DPPD is among the double treatments, that is, when DPPD was combined with one of the other additives. Statistically significant harmful effects on production are more evident, however, in the analysis of the data on the number of kits weaned. All of the lots that received DPPD show reduced numbers.

Analysis of variance of the weaning weights (table 8) shows highly significant differences among treatments and replicates. When this analysis of the treatment means is broken down into orthogonal comparisons among the double treatments, the group that received

TABLE 5.—DIETS: *Experiment 2*

Basal diet (all lots)		Parts
Pink salmon waste-----		
Dry mix No. 3 <sup>1</sup> -----		
Wheat germ meal-----		
Brewers' yeast-----		
Liver meal-----		
Water-----		
Total-----		
Additives		Quantity
Toopherol <sup>2</sup> -----	III, IV, VII, VIII-----	1.2 lbs. per ton of feed.
Antibiotic <sup>3</sup> -----	V, VI, VII, VIII-----	1.6 lbs. per ton of feed.
Antioxidant <sup>4</sup> -----	II, IV, VI, VIII-----	112 gm. per ton of feed.
Wheat germ oil <sup>5</sup> -----	Each lot-----	5 gm. per day.

<sup>1</sup> See Appendix, p. 23.

<sup>2</sup> A commercial grade labeled to contain 20,000 International Units of vitamin E per pound as alpha tocopherol acetate.

<sup>3</sup> Labeled to contain not less than 5 grams of chlorotetracycline (CTC) per pound.

<sup>4</sup> Diphenyl-p-phenylene diamine (DPPD).

<sup>5</sup> Wheat germ oil was added to the daily ration of each lot from Feb. 1 until Apr. 1.

TABLE 6.—PRODUCTION: *Effect of feed additives, tocopherol, DPPD, and CTC, Experiment 2*

Traits measured	Lot No. and additive							
	I Control	II DPPD	III TOCOL	IV TOCOL DPPD	V CTC	VI CTC DPPD	VII CTC TOCOL	VIII CTC TOCOL DPPD
Females:								
Started on experiment-----	12	12	12	12	12	12	12	12
Mated-----	11	12	12	12	12	12	12	11
Producing-----	10	7	9	7	10	6	11	11
Litters destroyed-----	1	1	2	1	0	1	2	5
Kits:								
Total:								
At 10 days of age-----	45	25	30	25	38	20	48	17
Died between 10 days and weaning-----								248
do-----	1	0	0	5	1	1	7	2
Weaned at 50 days-----	44	25	30	20	37	19	41	15
Average:								
At 10 days per female started-----	3.75	2.08	2.50	2.08	3.16	1.66	4.00	1.41
Weaned per female started-----	3.66	2.08	2.50	1.66	3.08	1.58	3.41	2.58
Weaned per producing female-----	4.40	3.57	3.33	3.00	3.70	3.16	3.72	3.25
Weaning weight at 50 days of age pounds-----	.686	.731	.738	.635	.707	.709	.791	.812
								.725

TABLE 7.—*Chi-square studies of effects of treatment variables on production, Experiment 2*

Comparisons	Degrees of freedom	X <sup>2</sup> tests of significance among treatments <sup>1</sup>	
		Number of females producing	Number of kits weaned
Control vs. treated-----	1	1. 531	9. 054**
Among single treatments-----	2	1. 778	2. 370
Among double treatments-----	2	5. 336	11. 575**
DPPD-TOCOL vs. DPPD-CTC-----	1	. 190	. 019
DPPD-TOCOL and DPPD-CTC vs. CTC-TOCOL	1	5. 145*	11. 556**
Single vs. double treatments-----	1	. 254	. 837
Single and double vs. triple treatments-----	1	3. 630	5. 993*

<sup>1</sup> 1 asterisk indicates significance at the 5-percent level; 2 asterisks indicate significance at the 1-percent level.

DPPD-TOCOL shows depressed growth. When this was supplemented with the antibiotic (lot VIII), increased body weights were obtained. But other factors, such as greater milk supply due to fewer kits, may have produced this effect.

#### *Incidence of Steatitis*

As in Experiment 1, in order to reduce the probability of high death losses from steatitis of many valuable animals, it was necessary to discontinue lots I (Control) and V (CTC) in July. Six death losses occurred in lot I and six in lot V. The fish waste diets were continued in the other lots, and before November, three losses occurred in lot III (TOCOL) and one in lot VII (CTC-TOCOL). No losses occurred in the four lots that received DPPD up to November 20. At this time it was becoming well established that DPPD could result in adverse effects in reproduction in animals. Since the supply of DPPD being used in this work was exhausted, it

was decided to continue the fish waste diets without the DPPD supplement for the remaining 2 weeks of the experiment. Within less than 1 week, losses occurred in lots II and IV from what was considered very severe steatitis. The animal carcasses showed an acute edemic condition due to decomposition and separation of fatty tissue. At pelting many of the skins showed this condition.

Thus the effects of DPPD were somewhat obscured by this development in the last 2 weeks of the experiment; however, there were no signs of, or losses from, steatitis in any of the 4 lots that received DPPD during the period it was being fed. There were losses in all four lots that were not fed DPPD during the same period. The immediate response of these animals to withdrawal of the protective antioxidant is of interest as it emphasizes the sensitiveness of the animals to the fish waste and control of steatitis by the antioxidant.

TABLE 8.—*Analysis of variance of weaning weights of kits, Experiment 2*

Source of variation	Degrees of freedom			Sums of squares			Mean square 1		
	Replicates	Treatments	Error	11	7	210	0.6055	.3472	3.7133
Orthogonal comparisons									
Control vs. treated				1			0.0526		0.0526
Among single treatments				2			.0182		.0091
DPPD vs. TOCOL and CTC				1			.0169		.0169
TOCOL vs. CTC				1			.0007		.0007
Among double treatments				2			.2125		.1062*
DPPD-TOCOL vs. DPPD-CTC				1			.0238		.0238
DPPD-TOCOL and DPPDC-TC vs. CTC-TOCOL				1			.1780		.1780**
Single vs. double treatments				1			.0328		.0328
Single and double vs. triple treatments				1			.0500		.0500
Error				210			3.7133		.0177
Lot No. and additive									
I	II	III	IV	V	VI	VII	CTC DPPD	CTC TOCOL	CTC TOCOL DPPD
Control	DPPD	TOCOL	TOCOL DPPD	CTC	CTC DPPD	CTC TOCOL			
Least squares means--pounds--	0.731	0.759	0.787	0.689	0.794	0.744	0.814	0.833	

\* 1 asterisk indicates significance at the 5-percent level; 2 asterisks indicate significance at the 1-percent level.

## FEEDING EXPERIMENTS WITH BUTYLATED HYDROXY TOLUENE (BHT)

Feeding-reproduction experiments were continued throughout a 3-year period with BHT. The feeding level in all experiments was at 112 grams per ton of wet feed (0.0125 percent), which is only a little more than twice the tolerance level of 50 p.p.m. permitted under the additive law enacted in 1959 for use in human food. This means that the compound has at least undergone testing with laboratory animals and thus far no harmful effects have been reported.

## EXPERIMENT 3

Pink salmon waste, even at 50 percent, would be expected to result in development of some steatitis under the conditions of these tests, but the comparative effects of other fish types with and without an antioxidant is of interest.

Experiment 3 was designed to compare fish waste from three fish types (pink, chum, and red salmon) with and without BHT in the diet. Wheat germ meal, yeast, liver meal, and an antibiotic were included as constant additives in all diets (table 9).

TABLE 9.—DIETS: *Experiment 3*

Basal mixture (all lots)		Parts
Flounders-----		25
Dry mix No. 3 <sup>1</sup> -----		10
Brewers' yeast-----		2
Wheat germ meal-----		2
Liver meal-----		1
Water-----		10
Total-----		50
Fish waste		Parts
Pink salmon-----	I, II-----	50
Chum salmon-----	III, IV-----	50
Red salmon-----	V, VI-----	50
Total-----	Each lot-----	50
Additives		Quantity
Antioxidant <sup>2</sup> -----	II, IV, VI-----	112 gm. per ton of feed.
Antibiotic <sup>3</sup> -----	Each lot-----	1.4 lbs. per ton of feed.
Wheat germ oil <sup>4</sup> -----	do-----	5 gm. per lot per day.

<sup>1</sup> See Appendix, p. 23.

<sup>2</sup> Butylated hydroxy toluene (BHT).

<sup>3</sup> Labeled to contain not less than 5 gm. of chlorotetracycline (CTC) per lb.

<sup>4</sup> Wheat germ oil was added to the daily ration of each lot from Feb. 1 until Apr. 1.

## *Production and Growth of Kits*

Complete breeding and production results from the animals on this experiment are given by lots in table 10.

Production was considered below standard largely because 19 of the females (20 percent) bred but failed to produce. Five of the breeding failures were attributed to the use of one sterile male.

Statistical analyses of the data on the number of females producing and number of kits weaned (table 11) result in chi-square values which indicate no significant differences

due to the treatments in either of the traits studied.

Analysis of variance of weaning weights of the kits shows highly significant differences among the replicates and treatments (table 12). Least squares means of the adjusted weight gains show higher weights in the groups receiving BHT. When the analysis was extended to make orthogonal comparisons (table 12), a highly significant mean square was obtained for the comparison of groups with and without BHT. However, data from Experiments 4 and 5, presented in the next section of this report, fail to verify this effect.

TABLE 10.—PRODUCTION: *Effect of butylated hydroxy toluene and different fish types, Experiment 3*

Traits measured	Fish waste type, lot No., and additive						Total or average, all lots	
	Pink salmon		Chum salmon		Red salmon			
	I None	II BHT	III None	IV BHT	V None	VI BHT		
<b>Females:</b>								
Started on experiment number	16	16	16	16	16	16	96	
Mated do	14	14	16	16	15	16	91	
Producing do	11	12	10	13	14	12	72	
Litters destroyed do	1	1	1	1	2	2	8	
<b>Kits:</b>								
Total:								
At 10 days of age do	49	48	44	44	58	35	278	
Died between 10 days of age and weaning do	1	0	0	6	2	0	9	
Weaned at 50 days do	48	48	44	38	56	35	269	
<b>Average:</b>								
At 10 days per female started do	3.06	3.00	2.75	2.75	3.62	2.19	2.90	
Weaned per female started do	3.00	3.00	2.75	2.37	3.50	2.19	2.80	
Weaned per producing female do	4.36	4.00	4.40	2.92	4.00	2.92	3.74	
Weaning weight at 50 days of age pounds	.831	.828	.803	.884	.787	.966	.841	

TABLE 11.—*Chi-square studies of effects of treatment variables on production, Experiment 3*

Comparisons	Degrees of freedom	X <sup>2</sup> tests of significance among treatments	
		Number of females producing	Number of kits weaned
Among salmons-----	2	2. 653	1. 514
Among salmons plus BHT-----	2	. 204	2. 298
Between supplemented and nonsupplemented salmons-----	1	. 204	2. 710

TABLE 12.—*Analysis of variance of weaning weights of kits, Experiment 3*

Source of variation	Degrees of freedom	Sums of squares	Mean square <sup>1</sup>			
Replicates-----	15	1. 5794	0. 1053**			
Treatments-----	5	. 9429	. 1886**			
Error-----	247	12. 0781	. 0489			
Orthogonal comparisons						
Among salmons-----	2	0. 1001	0. 0500			
Chum vs. pink and red-----	1	. 0383	. 0383			
Pink vs. red-----	1	. 0565	. 0565			
Among salmons plus BHT-----	2	. 3228	. 1614*			
Chum plus BHT vs. pink plus BHT and red plus BHT-----	1	. 0037	. 0037			
Pink plus BHT vs. red plus BHT-----	1	. 3225	. 3225*			
Between supplemented and nonsupplemented salmons-----	1	. 5881	. 5881**			
Error-----	247	12. 0781	. 0489			
Fish waste type, lot No., and additive						
Pink salmon		Chum salmon	Red salmon			
I None	II BHT	III None	IV BHT	V None	VI BHT	
Least squares means-----pounds	0. 822	0. 872	0. 809	0. 926	0. 874	1. 005

<sup>1</sup> 1 asterisk indicates significance at the 5-percent level; 2 asterisks indicate significance at the 1-percent level.

### *Incidence of Steatitis*

None of the animals that received chum salmon waste developed steatitis (table 13). The animals fed diets containing red and pink salmon with

no antioxidant were affected to varying degrees by the disease. The one slight case noted among animals protected by BHT is atypical, as shown by this experiment and Experiments 4 and 5.

TABLE 13.—*Incidence and degree of steatitis, Experiment 3*

Lot No.	BHT per ton of feed	Kits Dec. 10	Pelted	Cases of steatitis at pelting				Number
				Slight	Medium	Extreme	Total	
I-----	Grams 0	Number 22	Number 16	Number 4	Number 3	Number 4	Number 11	
II-----	112	21	14	1	0	0	0	1
III-----	0	28	19	0	0	0	0	0
IV-----	112	21	21	0	0	0	0	0
V-----	0	30	21	6	1	1	8	
VI-----	112	18	11	0	0	0	0	0
All lots-----		140	102	11	4	5	20	

### EXPERIMENTS 4 AND 5

Results from Experiment 3 indicated that BHT was not affecting reproduction at the level used, but the number of animals in the experiment was small. Also, Experiment 2 had indicated that females receiving DPPD for the second year may have demonstrated residual effect that caused reduced reproduction in this second year. Therefore, further data were needed to increase

confidence in the safety of the use of BHT.

Experiments 4 and 5 conducted during 1958 and 1959, were a repetition of Experiment 3.

The same diets and procedures were used in all three experiments (table 9). Data on reproduction and statistical studies and incidence of steatitis for Experiments 4 and 5 are given in tables 14 through 21.

TABLE 14.—PRODUCTION: *Effect of butylated hydroxy toluene and different fish types, Experiment 4*

Traits measured	Fish waste type, lot No., and additive						Total or average, all lots	
	Pink salmon		Chum salmon		Red salmon			
	I None	II BHT	III None	IV BHT	V None	VI BHT		
Females:								
Started on experiment number—	16	16	16	16	16	16	96	
Mated—do—	16	16	15	16	16	15	.94	
Producing—do—	13	12	13	14	13	11	.76	
Litters destroyed number—	2	1	0	3	1	1	8	
Kits:								
Total:								
At 10 days of age number—	38	44	48	45	57	46	278	
Died ten days of age to weaning number—	0	15	0	0	14	1	10	
Weaned at 50 days number—	38	39	48	45	53	45	268	
Average:								
At 10 days per female started number—	2.37	2.75	3.00	2.81	3.56	2.87	2.90	
Weaned per female started number—	2.37	2.45	3.00	2.81	3.31	2.81	2.79	
Weaned per producing female number—	2.92	3.25	3.69	3.21	4.08	4.09	3.53	
Weaning weight at 50 days of age pounds—	.772	.792	.828	.754	.862	.766	.802	

<sup>1</sup> 4 of the 5 kits in lot II and 1 of the 4 kits in lot V were entire litters.

TABLE 15.—*Chi-square studies of effects of treatment variables on production, Experiment 4*

Comparisons	Degrees of freedom	X <sup>2</sup> tests of significance among treatments	
		Number of females producing	Number of kits weaned
Among salmons	2	0.000	2.5180
Among salmons plus BHT	2	.769	.5581
Between supplemented and nonsupplemented salmons	1	.252	.3731

TABLE 16.—*Analysis of variance of weaning weights of kits, Experiment 4*

Source of variation	Degrees of freedom	Sums of squares	Mean square <sup>1</sup>			
Replicates -----	15	1. 0697	0. 0713**			
Treatments -----	5	. 4580	. 0916**			
Error -----	246	4. 6343	. 0188			
Orthogonal comparisons						
Among salmons-----	2	0. 2405	0. 1202**			
Chum vs. pink and red-----	1	. 0018	. 0018			
Pink vs. red-----	1	. 2320	. 2320			
Among salmons plus BHT-----	2	. 0781	. 0390			
Chum plus BHT vs. pink plus BHT and red plus BHT-----	1	. 0642	. 0642*			
Pink plus BHT vs. red plus BHT-----	1	. 0022	. 0022			
Between supplemented and non-supplemented salmons-----	1	. 1341	. 1341**			
Error -----	246	4. 6343	. 0188			
Fish waste type, lot No., and additive						
Pink salmon		Chum salmon	Red salmon			
I None	II BHT	III None	IV BHT			
V None	VI BHT					
Least squares means-----pounds-----	0. 831	0. 860	0. 879	0. 804	0. 942	0. 848

<sup>1</sup> 1 asterisk indicates significance at the 5-percent level; 2 asterisks indicate significance at the 1-percent level.

TABLE 17.—*Incidence and degree of steatitis as affected by BHT, Experiment 4*

Lot No.	BHT per ton of feed	Kits, Dec. 10	Pelted	Cases of steatitis at pelting			
				Slight	Medium	Extreme	Total
I-----	0	17	13	1	3	4	8
II-----	112	20	13	0	0	0	0
III-----	0	32	27	0	0	0	0
IV-----	112	27	21	0	0	0	0
V-----	0	33	24	9	5	8	22
VI-----	112	19	11	0	0	0	0
All lots-----		148	109	10	8	12	30

TABLE 18.—PRODUCTION: *Effect of butylated hydroxy toluene and different fish types, Experiment 5*

Traits measured	Fish waste type, lot No., and additive						Total or average, all lots	
	Pink salmon		Chum salmon		Red salmon			
	I None	II BHT	III None	IV BHT	V None	VI BHT		
Females:								
Started on experiment number	16	16	16	16	16	16	96	
Mated do	16	16	16	16	16	16	96	
Producing do	14	14	13	16	15	15	87	
Litters destroyed number	1	0	4	2	1	2	10	
Kits:								
Total:								
At 10 days of age number	63	66	42	70	74	65	380	
Died 10 days of age to weaning number	6	4	1	16	16	14	27	
Weaned at 50 days number	57	62	41	64	68	61	353	
Average:								
At 10 days per female started number	3.94	4.125	2.625	4.375	4.625	4.06	3.958	
Weaned per female started number	3.56	3.875	2.56	4.00	4.25	3.81	3.677	
Weaned per producing female number	4.07	4.428	3.153	4.00	4.533	4.066	4.057	
Weaning weight at 50 days of age pounds	.787	.843	.881	.894	.821	.809	.837	

<sup>1</sup> All 6 kits in lot IV, 5 of the 6 in lot V, and 3 of the 4 in lot VI were entire litters.

TABLE 19.—*Chi-square studies of effects of treatment variables on production, Experiment 5*

Comparisons	Degrees of freedom	X <sup>2</sup> tests of significance among treatments <sup>1</sup>	
		Number of females producing	Number of kits weaned
Among salmons			
Chum vs. pink and red	2	1.471	6.663*
Pink vs. red	1	( <sup>2</sup> )	5.569*
Among salmons plus BHT	1	( <sup>2</sup> )	1.093
Between supplemented and nonsupplemented salmons	2	1.471	.075
	1	1.103	1.249

<sup>1</sup> 1 asterisk indicates significance at the 5-percent level.

<sup>2</sup> Not calculated.

TABLE 20.—*Analysis of variance of weaning weights of kits, Experiment 5*

Source of variation	Degrees of freedom	Sums of squares	Mean square <sup>1</sup>			
Replicates.....	15	1. 9035	0. 1269			
Treatments.....	5	. 8705	. 1741			
Error.....	331	26. 4970	. 0801			
Orthogonal comparisons						
Among salmons.....	2	0. 4056	0. 2028			
Chum vs. pink and red.....	1	. 4003	. 4003*			
Pink vs. red.....	1	. 0133	. 0133			
Among salmons plus BHT.....	2	. 4287	. 2144			
Chum plus BHT vs. pink plus BHT and red plus BHT.....	1	. 2932	. 2932			
Pink plus BHT vs. red plus BHT.....	1	. 1356	. 1356			
Between supplemented and non-supplemented salmons.....	1	. 0777	. 0777			
Error.....	331	26. 4970	. 0801			
Fish waste type, lot No. and additive						
		Pink salmon	Chum salmon	Red salmon		
	I None	II BHT	III None	IV BHT	V None	VI BHT
Least squares means—pounds—	0. 830	0. 816	0. 697	0. 867	0. 809	0. 747

<sup>1</sup> 1 asterisk indicates significance at the 5-percent level.

TABLE 21.—*Incidence and degree of steatitis, Experiment 5*

Lot No.	BHT per ton of feed	Kits, in lots	Pelted	Cases of steatitis at pelting				Number
				Slight	Medium	Extreme	Total	
I.....	Grams 0	Number 29	Number 25	Number 2	Number 4	Number 5	Number 11	11
II.....	112	31	26	0	0	0	0	0
III.....	0	23	21	0	0	0	0	0
IV.....	112	28	27	0	0	0	0	0
V.....	0	37	33	8	0	0	0	8
VI.....	112	34	28	0	0	0	0	0
All lots.....		182	160	10	4	5	19	

## *Production and Growth of Kits*

Reproduction on Experiments 4 and 5 (tables 14 and 18) shows considerable contrast. Results were better in Experiment 5 than in Experiment 4. This evidently is not a result of treatment variables but is due to uncontrolled variables. In any event, the good production in Experiment 5 is important evidence that BHT had no residual toxic effect in the mink herd after its use during a 3-year period.

Statistical studies of the production from both experiments (tables 15 and 19) again show that the numbers of females producing and the numbers of kits weaned are not affected significantly by the level of BHT fed in these experiments. The analysis of variance of kit weaning weights from Experiment 4 indicates significantly lower weights of kits fed the supplemented salmon waste, but this is in contrast to results obtained in Experiment 3. Further, the corresponding data from Experiment 5 fail to indicate any differences in kit weaning weights resulting from intake of BHT at the levels fed in these experiments.

### *Incidence of Steatitis*

The results obtained in both Experiments 4 and 5 (tables 17 and 21) agree with the results obtained in Experiment 3. BHT prevented any development of steatitis in the animals receiving red or pink salmon waste. No steatitis was observed in animals fed chum salmon waste, but all lots in both experiments fed pink and red salmon and unprotected by BHT had some animals in various stages of the disease at pelting time.

Many experiments with antiox-

idants during recent years have shown that some substances act as synergists within the usually complicated system in which antioxidants are used. For this reason it may be difficult to predict how effective an antioxidant will be. However, BHT appeared to be an effective antioxidant in preventing steatitis in mink, at the level fed in these experiments (0.0125 percent). It showed no immediate or residual adverse effects on reproduction in these animals.

## SUMMARY AND CONCLUSIONS

The availability of large amounts of fish cannery waste in the Alaska area as feed for minks and the susceptibility of this product to rancidity prompted tests on feed additives that might preserve this material. From previous experiments and practical mink feeding, it was known that stored frozen fish waste, fed at relatively high levels, resulted in steatitis (yellow fat disease) in minks. Also in these previous studies supplementary antibiotic or tocopherols at the levels used, though of some help, failed to prevent the disease.

The phenolic antioxidants, including DPPD and BHT, tocopherol, and an antibiotic, were tested as additives to fish waste diets in factorially designed experiments. In these experiments, conducted during a 5-year period, a total of 474 female minks and their 1,420 kits were fed the experimental diets through the breeding, gestation, and suckling periods. Many of the animals were fed to pelting.

Results of the experiments during the first 2 years of this work with

the antioxidant DPPD gave rather conclusive evidence that DPPD, although effective as an antioxidant, causes adverse effects on mink reproduction even when fed at the relatively low level of 112 grams per ton of wet feed (0.0125 percent).

In a factorial experiment the following year, 6 lots of 16 females each were used. Three of these lots, 48 females, received the antioxidant BHT at a level of 112 grams per ton of feed. Three types of fish waste were used: pink, chum, and red salmon. Each fish type was fed as 50 percent of the diet to a lot with and a lot without BHT.

The females fed through the reproductive period and half of the producing females and their kits fed to the pelting season in December demonstrated that BHT was effective in preventing steatitis on these diets. Also, differences in the salmons were shown because no steatitis appeared in the animals fed chum salmon whereas cases of the disease occurred in both lots fed the other salmons unprotected by BHT. Statistical studies of the data indicate no adverse effect on reproduction, but because of the limited number of animals used the experiment was repeated in both 1958 and 1959.

The previous conclusions were verified by both years' experiments. Results of the third experiment with BHT may be of more practical interest because reproduction was considered good throughout all the lots. The 96 females produced 380 kits, 201 kits from the 48 females receiving BHT and 179 kits from the 48 controls.

Statistical studies on this experiment indicated no significant differ-

ence due to BHT in the production of these kits or their weaning weights. BHT was quite effective in preventing steatitis in all lots of animals when fed at the 0.0125-percent level.

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## APPENDIX

### DIET MATERIALS<sup>4</sup>

#### Dry Mix No. 3

The dry mixture used in all of these experiments was prepared according to Experimental Fur Station specifications by Crown Mills, Portland, Oreg. This cereal mix was purchased in ton lots as needed to insure as fresh a supply as possible. The formula used for this mix is given in table 22.

TABLE 22.—*Dry mixture No. 3 used in Experiments 1 to 5*

Ingredient	Amount used
	Percent
Cooked whole wheat (dextrinized)	30
Cooked oat groats (dextrinized)	16
Wheat germ meal	10
Dried brewers' yeast	10
Beet pulp	5
Liver meal—commercial	5
Dried skim milk	15
Dehydrated grass, or alfalfa meal having a carotene content of 65 mg. or more per lb.	5
Ground limestone	3
2 lbs. Dry A (10,000 A per lb.) <sup>1</sup>	
1 lb. Dry D-3 (3,000 D per lb.) <sup>1</sup>	
7 lbs. wheat germ meal	0.5
Salt	0.5

<sup>1</sup> In Experiment 1 this vitamin supplement was supplied by 10 lbs. 1800 A, 175 D cod liver oil.

#### Description and Source of Other Ingredients

Brewers' yeast, wheat germ meal, and liver meal were purchased from Crown Mills as needed.

<sup>4</sup> Mention of a specific product or a commercial concern does not imply endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

The antibiotic<sup>5</sup> used in these experiments was Eurofac D containing not less than 5 grams Aureomycin (chlorotetracycline) per pound, Lederle Laboratories, and purchased from Crown Mills.

N,N'—Diphenyl-p-phenylene diamine (DPPD), Eastman, technical grade.

Butylated hydroxy toluene, Nopco Chemical Company, agricultural grade. Supplied gratis by manufacturer.

Tocopherol, "Myvamix" (a commercial grade vitamin E supplement containing distilled alpha-tocopherol acetate concentrate absorbed on soybean meal and containing 20,000 I.U. of vitamin E per pound), manufactured by and purchased from Distillation Products Industries, Rochester, N.Y.

#### FUR RATING AND FOOD CONSUMPTION

A hundred or more minks were pelleted from each of the experiments described. The pelts were shipped to the Seattle Fur Exchange for examination, grading, and subsequent sale at public auction.

In most of the experiments, because of the large difference in numbers, sex, and color phase between the animals in various lots, it was very difficult to get a good comparison of the individual lots due to diet treatments. Generally these mink skins lacked quantity of fur. They were short furred and weak on coverage. This criticism was particularly true

<sup>5</sup> Although this material contributed additional vitamin B<sub>12</sub> to the diets, this was thought not to be a factor in the experiments.

of the dark and pastel pelts with the few palomino skins being the best in this respect. The color of the skins was satisfactory and the size was equal to or better than that on most ranches raising these types of minks.

Considerable evidence indicates that the poor quality (short and low-density fur) of the skins pelted from animals raised on Experiments 1, 2, 3, and 4 was due, at least in part, to the character of the original Station breeding stock. This stock had been inbred for nearly 20 years. A small number (20 females and 7 males) of good quality breeders purchased in 1958 and bred with the Station stock in Experiment 5 resulted in much better pelts. The three cotton skins produced in Experiment 5 were all from animals raised on the control pink salmon waste ration (lot I) and did not receive the antioxidant.

In the experiments comparing the salmon types, the pelts from lots III and IV (chum salmon waste) were judged best and from lots I and II (pink salmon waste) were poorest. Some

cotton and weak-hipped skins occurred in these poorer lots.

There was no evidence to indicate that the antioxidants affected pelt quality except insofar as they modified health by controlling steatitis. For this reason it appears that a full record of the data on fur results would not contribute materially to this report.

Complete feed consumption records were kept by lots rather than by individual animals. These feed records usually reflected general health of the animals but were not adapted to statistical analysis and appear to offer little that would contribute to this report.

#### ADJUSTMENT FACTORS

Factors used to adjust weaning weights of females to a male basis are as follows:

Experiment	Pounds
1	0.069
2	.100
3	.137
4	.135
5	.143



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